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CHEMICAL PROFILE ANALYSIS AND ANTI-INFLAMMATORY ACTIVITY OF POLAR FRACTION FROM SOYBEANS (*GLYCINE MAX*)

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KEYWORDS: *Glycine max* soybean, polar components, HPLC fingerprint, anti-inflammation

INTRODUCTION

Presently, phytochemicals from soybeans have received more attention. They are used as neutraceutical ingredients due to their numerous health beneficial effects. Soy saponins, the main polar components, which exhibited *in vitro* antioxidation and anti-inflammation (1-4), as well as cytotoxicity to several cancer cell lines such as Hep-G2(5), Hela (6) and CaCo-2 (7-8) are among of interest. In this study, we investigated on chemical profile of soybean's polar fraction and its *in vivo* anti-inflammatory activity. HPLC-PDA-ELSD was employed for the chemical fingerprint analysis.

MATERIALS AND METHODS

Plant material: Soy beans were purchased from health food shop (Lemon green) in Bangkren District, Bangkok, in October 2011. They were pulverized into coarse powder (20 mesh), then exhaustedly soxhletted with hexane to give the defatted soy bean powder (DFP).

Extraction and fractionation DFP 1.1 kg were macerated with 11 L of 70% ethanol for 72 h with occasional stirring, then filtered. After removal of ethanol under reduced pressure, 300 ml portion of aqueous solution equivalent to 360 g of DFP was extracted with 3x200 ml of *n*-butanol. The combined *n*-butanol solution was concentrated under reduced pressure to give 4.9 g of dried extract (BuFr).

HPLC analysis (9) BuFr was dissolved in methanol (1mg/ml) and PTFE 0.45 μm filtered, then subjected to HPLC analysis. Confirmation of components was done by comparision of Rt and absorbance spectral with reference standards.

Analysing condition

HPLC: Waters Pump600

Column: Nova-Pak C_{18} (150 mm x 3.9 mm i.d., particle size 4 μm)

Mobile phase: 0.1% trifluoroacetic acid in water (A) and acetronitrile (B), gradient elution: 100A to 62A/ 38B for 20 min, 62A/ 38B isocratic for 5 min, 62A/ 38B to 87A/ 13B for 25 min.

Flow rate: 0.8 ml/min

Inject volume: 10 μl

Detection: Photo diode array (PDA) at 205 nm and evaporative light scattering detector (ELSD) at probe temperature 50°C, a gain of 6.0 and nebulizer nitrogen gas of 3.3±0.1 bar.

Reference standards:

Soy isoflavones: daidzin, glycitin,genistin, daidzein, glycitein, genistein (Chromadex USA)

Soy saponins: soyasaponin I (Chromadex USA)

Anti-inflammatory Test (10)

Animals Male Wistar rats were purchased from Laboratory Animal Centre, Mahidol University, Salaya, Nakornprathom, Thailand. The animals were housed in the animal facility of Thailand Institute of Scientific and Technological Research under standard conditions (25±2°C), 50-60% of humidity and 12 h/ 12 h light/dark cycles. The animals were kept under laboratory conditions for one week prior to the start of the experiments. Food and water were allowed *ad libitum*.

Carrageenan induced paw oedema Rats weighing 80-100 g were divided in groups of six: vehicle control (distilled water), positive control (diclofinac 50 mg/kg), test sample I (BuFr 100 mg/kg) and test

sample II (BuFr 200 mg/kg). At the beginning of the experiment, initial paw volumes were determined using a water plethysmometer (Ugo Basil, Italy). Then, individual animal group orally received sample accordingly. One hour after sample administration, paw oedema was induced by injection of 0.1 ml of carrageenan (λ-carrageenan, type IV, Sigma) diluted in saline in the right hind foot pad. Paw volumes were determined at times 1, 2, and 3 h after oedema induction. The percentage of oedema inhibition was calculated with reference to vehicle control group.

Analysis of Data The results are expressed as means ± S.E.M. Differences in mean values between groups were analyzed by a one-way analysis of variance (ANOVA).

RESULTS

HPLC analysis HPLC chromatogram of BuFr detected under PDA at 205 nm (Fig. 1D) showed the presence of isoflavones daidzin, glycitin, genistin, daidzein, glycitein and genistein at Rt of 16.09, 16.90, 20.66, 29.70, 30.58 and 32.66, respectively as confirmed by Rt of standard isoflavones (Fig. 1A). Peak corresponding to soyasaponin I was observed at Rt 39.28 confirmed by ELSD (Fig. 1B, Fig. 1C). Among isoflavone constituents, genistein appeared to be the main component followed by genistin and daidzein, respectively.

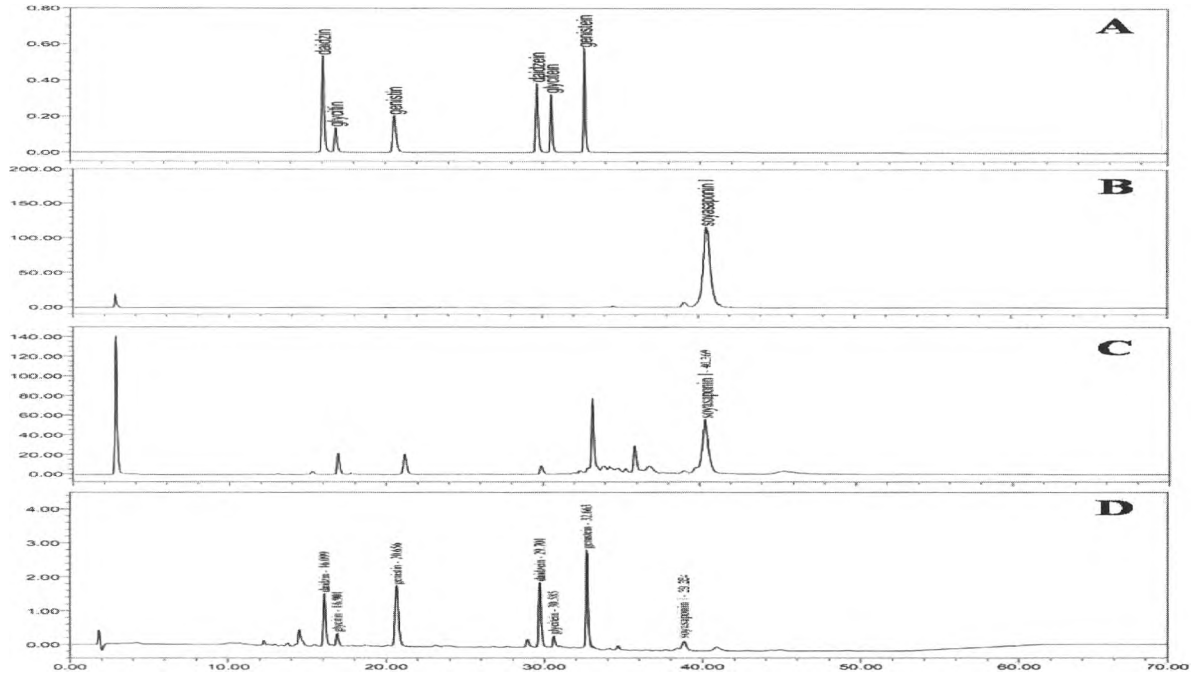

Figure 1 HPLC Chromatograms of polar fraction from soybeans (BuFr) and reference standards.
(A) standard isoflavones, PDA 205 nm, (B) soyasaponin I, ELSD, (C) BuFr, ELSD, (D) BuFr, PDA 205nm.

Anti-inflammatory activity BuFr at 100 and 200 mg/kg p.o. exhibited oedema inhibitory effect at time 2 and 3 h similar to that of diclofinac 50 mg/kg (Table 1). However, the effect seemed to be less potent and less lasting than diclofinac. Maximum effect of BuFr was observed at 2h while that of diclofinac appeared to be not less than 3 h. Interestingly, the anti oedema effect of BuFr was dose dependent manner at time 2 and 3 h.

Table 1 Oedema inhibitory effect of polar fraction, BuFr from soybeans tested by carrageenan induced paw oedema in rat.

Samples	% Oedema			Oedema inhibition (%)		
	1 h	2 h	3 h	1 h	2 h	3 h
Control	31.06±2.27	63.40±7.84	76.17±7.24	-	-	-
Diclofenac 50 mg/kg	23.44±1.93*	20.92±3.60*	19.53±3.36*	24.00	64.00*	68.00*
BuFr 100 mg/kg	38.66±2.24*	22.25±3.73*	23.78±3.81*	6.00	40.00*	23.00*
BuFr 200 mg/kg	25.68±3.07*	31.23±3.21*	49.15±4.07*	9.00	51.00*	41.00*

n = 6, *significant from control, $P < 0.05$

% Swelling $= \frac{(T_t - T_0) \times 100}{T_0}$

T_0 = Paw volume before induced oedema

T_t = Paw volume after sample application and induced oedema

DISCUSSION

Chemical profile of polar fraction from defatted soybeans showed the presence of the representative phytochemicals including isoflavones and saponins. Seven components could be characterized using HPLC-PDA-ELSD including daidzin, glycitin, genistin, daidzein, glycitein, genistein and soyasaponin I. Structurally, there have been no UV active chromophores in saponin molecule. Thus, PDA detector at 205 nm gave a low intensity peak of soyasaponin I while the saponin selective ELSD detector gave higher intensity peak. In considering only saponin components, ELSD is recommended.

In vivo anti-inflammatory activity was selected for our preliminary pharmacological assessment of BuFr since many pathological disorders are associated with inflammation. BuFr at 100-200 mg/kg exhibited a moderate anti-inflammatory activity compared to the standard anti-inflammatory drug, diclofmac at 50 mg/kg. The result is in accordance with its chemical profile and previous *in vitro* anti-inflammatory activity (1-2). So BuFr is interesting to be used as a natural nutraceutical ingredient for anti-inflammation purpose.

CONCLUSION

n-Butanol fraction from the 70% aqueous ethanolic extract from defatted soybeans comprised both isoflavones and saponins detected by HPLC-PDA-ELSD. Seven components could be characterized from HPLC-PDA profile at 205 nm including daidzin, glycitin, genistin, daidzein, glycitein, genistein and soyasaponin I. The fraction given orally at 100 and 200 mg/kg showed *in vivo* anti-inflammatory effect tested by carrageenan induced rat paw oedema.

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REFERENCES

1. LY Z, et al. 2011. Anti-inflammatory effect of soyasaponins through suppressing nitric oxide production in LPS-stimulated RAW 264.7 cells by attenuation of NF-κB-mediated nitric oxide synthase expression. Bioorg Med Chem Lett. 15;2(8): 2415-8.
2. Kang JH, et al. 2005. Soybean saponins suppress the release of proinflammatory mediators by LPS-stimulated peritoneal macrophages. Cancer Lett. 18;230(2): 219-27.

3. Lee IA, Park YJ, Joh EH and Kim DH. 2011. Soyasaponin Ab ameliorates colitis by inhibiting the binding of lipopolysaccharide (LPS) to Toll-like receptor (TLR) 4 on macrophages. J Agric Food Chem. 28;59(24):13165-72.
4. Lee IA, Park YJ, Yeo HK, Han MJ and Kim DH. 2010. Soyasaponin I Attenuates TNBS-Induced Colitis in Mice by Inhibiting NF-κB Pathway. J Agric Food Chem. 58(20): 10929-34.
5. Zhang W, Popovich DG. 2008. Effect of soyasapogenol A and soyasapogenol B concentrated extracts on HEP-G2 cell proliferation and apoptosis. J Agric Food Chem 56(8):2603-8.
6. Xiao JX, et al. 2007. Morphological study on apoptosis Hela cells induced by soyasaponins. Toxicol In Vitro. 21(5): 820-6.KIM HY. 2004. Antiproliferative crude soy saponin extract modulates the expression of iKBa, protein kinase C, and cyclooxygenase-2 in human colon cancer cells. Cancer letters 210:(1)1: 1-6.
7. Ellington AA, Berhow M and Singletary KW. 2005. Induction of macroautophagy in human colon cancer cells by soybean B-group triterpenoid saponins. Carcinogenesis 26(1): 159-67.
8. Kang JH, et al. 2008. Soybean saponin inhibit tumor cell metastasis by modulating expressions of MMP-2, MMP-9 and TIMP-2. Cancer letters 261:1(8): 84-92.
9. Hamburger M, Slacanin I and Hostettmann K. 1992. Acelyted saponins with molluscicidal activity from Sapindus rarak:Unambiguous structure determination by proton nuclear magnetic resonance and quantitative analysis. Phytochemical analysis 3:231-237.
10. Winter CA, Edwin A, Risley and Nuss GW. 1962. Carrageenan-induced edema in hind paw of the rat as an assay for anti-inflammatory drugs. Proc. Soc. Exp. Biol. 111: 544-547.